REMARKS

I. Preliminary Remarks

Claims 30-33 as amended are currently pending. Claims 1-29 were canceled by previous amendments in the parent application. For the Examiner's convenience, a copy of the pending claims is attached hereto as Exhibit A.

Applicants acknowledge with thanks the interview kindly granted to Greta Noland, Applicants' agent, and Dr. Judith Woods, counsel for Applicants' assignee, on July 26, 1995. As indicated in the Examiner's Interview Summary Record, all grounds of rejection were discussed. The Examiner agreed to consider appropriate Declaration evidence and further written argument in support of patentability. While the Examiner suggested in the interview that the claims be amended to the application of the recited method in the treatment of bone marrow donors and the interview summary indicates that such amendment would be made, as a result of discussions with Applicants' expert declarants it became clear that the claims as proposed herein appropriately define the invention.

II. The Claimed Subject Matter

Claims 30-33 as amended are directed to a use of an antibody that binds to vascular cell adhesion molecule-1 (VCAM-1), in methods directly relating to Applicants' novel observations that bone marrow stromal cells express VCAM-1 and that VCAM-1 mediates adhesion between bone marrow stromal cells and bone marrow cells, especially those bearing the CD34 antigen. CD34 expression distinguishes a subset of bone marrow cells enriched in hematopoietic stem cells and progenitor cells. A representative embodiment of such an antibody that binds to VCAM-1 and that possesses the ability to block VCAM-1-mediated intercellular interactions is the 6G10 monoclonal antibody produced by hybridoma ATCC No. HB 10519.

III. The Outstanding Rejections

Claims 30-33 were rejected under 35 U.S.C. §112, first paragraph, for assertedly not enabling the breadth of the claimed invention; the Examiner questioned the *in vivo* operability of the invention and the therapeutic benefit of the treatment method.

Claims 30-31 were rejected under 35 U.S.C. §103 as unpatentable over Osborn et al., *Cell* 59:1203-1211, (1989) (hereafter "Osborn"), Elices et al., *Cell,* 60:577-584 (1990) (hereafter "Elices"), or Newman et al., U.S. Patent No. 5,011,778 (hereafter "Newman") in view of Graber et al., *J. Immunol.*, 145:819-830 (1990) (hereafter "Graber"), Rice et al., *Science, 246*:1303-1306 (1989) (hereafter "Rice (1989)"), Rice et al., *J Exp. Med.* 171:1369-1374 (1990) (hereafter "Rice (1990)"), Lewinsohn et al., *Blood* 75:589-595 (1990) (hereafter "Lewinsohn"), Shimizu et al., *Immunol. Rev.* 114:109-143 (1990) (hereafter "Shimizu"), or Prober et al., *Am. J. Pathol.* 133:426-433 (1988) (hereafter "Prober").

Claims 32-33 were rejected under 35 U.S.C. § 103 as unpatentable over Osborn, Elices or Newman in view of Graber, Rice (1989), Rice (1990), Lewinsohn, Shimizu or Prober as applied to claims 30-31 above and in further view of Knapp et al., Leukocyte Typing IV, Oxford Univ Press, pp. 1083, 1087 (1989) (hereafter "Knapp") and Hemler, *Immunol. Today* 9:109-113 (1988) (hereafter "Hemler").

IV. Patentability Arguments

A. The Rejection Under 35 U.S.C. §112, First Paragraph, May Properly Be Withdrawn

The rejection under 35 U.S.C. §112, first paragraph, may properly be withdrawn in light of the evidence provided in the accompanying Declaration of Thalia Papayannopoulou, M.D., Dr. Sci., Under 37 C.F.R. §1.132 (Exhibit B hereto) and the Declaration of Beverly J. Torok-Storb, Ph.D., Under 37 C.F.R. §1.132 (Exhibit C hereto).

It was the Examiner's position that persons of skill in the art would not believe the asserted *in vivo* operability of the claimed method in view of the general unpredictability

of pharmaceutical therapies and the absence of *in vivo* clinical data. These issues are addressed by the data provided in the Papayannopoulou Declaration, which show that systemic administration of an exemplary monoclonal antibody 6G10 to primates caused *in vivo* release of bone marrow progenitor cells from the bone marrow into the peripheral blood (see paragraph 8 of declaration). Briefly summarized, MAb 6G10 was administered to two non-human primates, whose levels of bone marrow progenitor cells in bone marrow aspiration samples and peripheral blood samples were assessed before, during and after therapy. The results showed that the concentrations of both erythroid burst-forming units (BFUe) and granulocyte/macrophage-colony-forming units (GM-CFU) rose significantly in the peripheral bloodstream after MAb 6G10 administration (see paragraphs 5 and 7 of declaration).

The Examiner also questioned what therapeutic benefits would be obtained from decreasing adhesion of bone marrow cells to bone marrow stromal cells. Specifically, the Examiner asked, "How do the claimed methods promote bone marrow transplantation or hemopoiesis?" In response to this query, Applicants submit herewith the Torok-Storb Declaration. In paragraph 4 of her declaration, Dr. Torok-Storb briefly describes the process of bone marrow transplantation. Bone marrow is harvested from the donor by direct aspiration from the bone. This bone marrow may be subjected to various procedures to render it enriched in primitive stem cells and hematopoietic progenitor cells. The recipient's immune system is destroyed to prepare the recipient for transplantation. This destruction is accomplished either through total body irradiation, chemotherapy (*e.g.*, cyclophosphamide treatment), or administration of anti-thymocyte globulin (which binds to and facilitates the destruction of the recipient's lymphocytes via the recipient's own complement system). The donor's bone marrow cells are then infused intravenously into the recipient's bloodstream.

In paragraph 6 of her declaration, Dr. Torok-Storb states that one of ordinary skill in the art, after being informed of the discovery of VCAM-1 expression on bone marrow stromal cells, would have understood from the disclosure in the application that a

clear therapeutic benefit of administering anti-VCAM-1 antibody to decrease adhesion of bone marrow cells to bone marrow stromal cells would be the interruption of progenitor/stroma binding. The consequential release of bone marrow cells into the bloodstream would allow those cells to be harvested directly from the blood of a donor. The advantages attendant upon this harvesting method are numerous: it is easier to harvest cells from blood than from bone marrow, the cells harvested are already conditioned to be in the blood (a desirable attribute because donor bone marrow is infused into the recipient's bloodstream), and the cells released are already enriched in primitive stem cells and progenitor cells.

Dr. Torok-Storb also notes that one of ordinary skill in the art would likely view an antibody-mediated decrease in bone marrow cell adhesion to be a therapeutic method even more easily employed in donors than in recipients, because the mere release of bone marrow cells is an adequate therapeutic endpoint with regard to harvest from the donor. In contrast, a standard but additional destructive step (destroying the immune-related bone marrow cells and other cells of the immune system using means known in the art) would need to accompany treatment of the recipient. In combination with such destructive means, the antibody-mediated release of bone marrow cells would also have been understood to have therapeutic benefit in recipients.

Finally, the Examiner stated that it was "not clear that the inhibition of stromal interactions with bone marrow cells occurs via VCAM per se or through other (cross-reactive) moieties recognized by the 6G10 antibody and expressed by stromal cells." In response, Applicants submit that the specific involvement of VCAM-1 in these adhesive interactions is confirmed by evidence that its major receptor, VLA4, is involved in the interaction between hemopoietic cells and the stroma. The accompanying article Papayannopoulou et al., "Peripheralization of hemopoietic progenitors in primates treated with anti-VLA_4 integrin," *Proc. Natl. Acad. Sci. USA* 90:9374-9378 (1993) (Exhibit D hereto), published after the effective filing date of this application, shows that the

administration of antibody to VLA4 selectively mobilized progenitor cells into the peripheral bloodstream.

In light of the declaration evidence showing the *in vivo* operability of the claimed invention and the clear therapeutic benefits of the intended use, the rejection under 35 U.S.C. §112, first paragraph, may properly be withdrawn.

B. The Rejection Under 35 U.S.C. §103 May Properly Be Withdrawn

The rejection of claims 30-31 under 35 U.S.C. §103 as unpatentable over Osborn, Elices or Newman in view of Graber, Rice (1989), Rice (1990), Lewinsohn, Shimizu or Prober may properly be withdrawn because *none of these references reports the existence of VCAM-1 on bone marrow stroma cells, and none of these references reports the existence of VLA-4 (the major receptor for VCAM-1) on bone marrow progenitor cells*. In the absence of such knowledge, the use of anti-VCAM-1 antibodies to decrease adhesion between bone marrow stromal cells and immature bone marrow cells cannot possibly have been suggested by the prior art.

The only cited reference that relates to bone marrow cells, Lewinsohn, does not teach that VLA4 is expressed on such cells.[1] In any case, a disclosure of VLA-4 expression on bone marrow cells would not teach that VCAM-1 is involved in stromal adhesion, because the VLA-4 could be binding to fibronectin in the stromal matrix rather than to VCAM-1. See Elices, M.J. et al., "VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site," *Cell,* 60:577-584 (1990) (document #O30).

Claim 30 has been amended to recite "an immature bone marrow cell" in response to the Examiner's concerns that the scope of Applicants' claims would encompass

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[1] Lewinsohn reports that H-CAM (CD44) is expressed on hematopoietic progenitor cells, and at page 594 merely states that "It will be of interest to ask whether the MEL-14 antigen and/or other putative lymphocyte adhesion systems are expressed on progenitor cells like H-CAM, and if so, what role they might play in interactions with stromal cells."

inhibiting a number of cells, including differentiated leukocytes, from adhering to a variety of cell types, including microvascular endothelial cells.

The rejection of claims 32-33 under 35 U.S.C. § 103 as unpatentable over Osborn, Elices or Newman in view of Graber, Rice (1989), Rice (1990), Lewinsohn, Shimizu or Prober as applied to claims 30-31 and in further in view of Knapp and Hemler may properly be withdrawn because the addition of Knapp and Hemler does not provide any teaching that VCAM-1 is involved in the interaction of bone marrow stromal cells with immature bone marrow cells.

The Examiner stated that Knapp and Hemler were added to provide evidence of the known widespread expression of CD34 and VLA-4 (CDw49d) at the time the invention was made. However, neither reference teaches that VLA-4 is expressed on immature bone marrow cells. Knapp merely notes that the "main cellular reactivity" of VLA-4α is with monocytes, T-cells and B-cells, and that "other reactive cells" include thymocytes, which are defined as "a lymphocyte arising in the thymus" according to Dorland's Illustrated Medical Dictionary, 26th ed., W. B. Saunders, page 1366 (1981) (Exhibit E hereto). Thus, Knapp does not disclose VLA-4α expression on immature bone marrow cells. With regard to Hemler, the Examiner acknowledged that "Hemler indicates that the bone marrow and thymus were not tested back in 1988," but maintained that "it was clear that this determination was under consideration at the time the invention was made." A suggestion to test bone marrow cells for VLA-4 expression is not a teaching that VLA-4 is expressed on these cells. In any case, a disclosure of VLA-4 expression on bone marrow cells would not teach that VCAM-1 is involved in stromal adhesion, because VLA-4 also binds to fibronectin.

In the absence of knowledge that VCAM-1 is expressed on bone marrow stromal cells and that VCAM-1 mediates adhesion of bone marrow stromal cells to immature bone marrow cells, particularly stem cells and progenitor cells, the prior art simply could not have suggested or provided a reasonable expectation of success for the claimed methods of

using antibody to VCAM-1. The rejection under 35 U.S.C. §103 thus may properly be withdrawn.

CONCLUSION

In light of the foregoing remarks, it is believed that claims 30-33 are now in condition for allowance, and early notice thereof is solicited.

Respectfully submitted,

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